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ABBREVIATED MORPHOMETRY REPORT WITH APPENDED THUMBNAILS OF SCANNED SECTIONS

PROTOCOL 1416-003

Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or *via* Maternal Milk

Supplement to Neuropathology Report on F_1 Generation Day 10 and Day 22 Postpartum Rats

TESTING FACILITY

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297

STUDY SPONSOR

Perchlorate Study Group
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Rancho Cordova, California 95670

February 22, 2001

11 Pages in Report

(Thumbnail Appendix Contains 78 Additional Pages)

Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or *via* Maternal Milk

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INTRODUCTION

A number of consultants have been asked to comment on the biologic significance of the brain morphometry data generated in the neuropathology component of this study. In order to assist these consultants, this supplemental report has been prepared to include thumbnail images of the measured brain sections. Although small in size, these thumbnails should help to at least establish the degree of section homology. Although printed in black and white, a CD bearing color images is also appended (in Photoshop® format). Larger-sized scans of each measured section can also be made available to the consultants on CD. However, because there are six of these CD's, they were not included with each of these reports.

MATERIALS AND METHODS

Animals and Study Overview

Crl:CD®(SD)IGS BR VAF/Plus® rats originating from Charles River Laboratories, Inc. were used for this study. The parental generation female rats had continual access to the test substance in the drinking water beginning 14 days before cohabitation and continuing through the day before sacrifice. There were five dosage groups, with these groups having the following target dosages: 0, 0.01, 0.1, 1.0, and 30.0 mg/kg/day. Necropsies were performed on rats at four age points, these being gestational age 21 and postpartum days 5, 10, and 22. This report presents small-sized images of the brain sections measured on the postpartum day (PND) 10 and 22 rats.

Dissection and Weighing of Brains

The F_1 generation rat pups examined on this study had been euthanatized by carbon dioxide asphyxiation. The heads of each of the pups had been severed just behind the back of the skull. The calvaria had then been removed from the top of each skull and the entire heads immersed in 10% neutral buffered formalin. These heads were subsequently shipped by the Testing Facility to either Consultants In Veterinary Pathology, Inc. (PND 10 rats) or to Experimental Pathology Labs, Inc. (PND 22 rats) where the brains were removed and weighed. Sixteen brains were to have been examined from each of the dose groups. However, due to necropsy damage, slightly fewer numbers are present in some groups.

Prior to slicing the brains for histologic processing, a Vernier caliper was used to obtain two anterior to posterior linear measurements from each brain as follows:

1) Anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs.
2) Anterior to posterior (AP) length of the cerebellum, extending from the anterior edge of the cerebellar cortex to the posterior pole (posterior edge of the cerebellar uvula). This measurement was made on a diagonal.

These gross measurements were performed in a "blinded" manner, with the individual performing the measurements being unaware of the dose group assignments.

After the gross measurements were taken, the brains were divided into six coronal sections as listed below.

Cut #1 - Half-way between the ventral base of the olfactory bulbs and the optic chiasm.
Cut #2 - Just anterior to the optic chiasm.
Cut #3 - Just anterior to the infundibulum.
Cut #4 - Through the midbrain at the level of the mammillary body.
Cut #5 - Through the cerebellum just anterior to its midpoint.
Cut #6 - Through the anterior portion of the medulla.

Cut #1 through Cut #4 were made from the ventral aspect of the brain, while Cuts #5 and #6 were made from the dorsal aspect of the brain. The olfactory bulbs were also processed, resulting in approximately eight slices from each brain. For all sections other than for the olfactory bulbs, the anterior face of each section was placed down into the tissue cassette, thus representing the face that was subsequently sectioned with the microtome. Each brain slice was approximately 2 mm in thickness.

The slice containing the optic chiasm was placed into Cassette No. 1. The slice that included the infundibulum was placed into Cassette No. 2. The slice through the mammillary body was placed in Cassette No. 3. The slice through the middle of the cerebellum was placed into Cassette No. 4. All of the remaining pieces of brain were placed into Cassette No. 5.

Histotechnology Procedures

The brain sections were processed according to the Standard Operating Procedures for paraffin embedding. The tissue blocks were then sectioned on a rotary microtome set at a thickness of six micrometers. The resulting sections from both the PND 10 and PND 22 brains were stained with hematoxylin and eosin (H&E). An additional set of sections from the PND 22 brains were also stained with the luxol fast blue/cresyl violet (LFB/CV) technique to better demonstrate regions that were myelinated. For the PND 22 rats, the morphometric measurements were performed on the LFB/CV-stained sections. The blocks representing cassettes No. 1 through 4 were step-sectioned and three to four sections from each block placed on each slide. (There are approximately 60 micrometers between each of these sections.) For Cassette No. 5, only one section was usually prepared. Sections were

initially prepared from rats in the control and high dose (Group V) groups. Subsequent to the initial evaluation of these sections, the blocks from the male and female rats in the other dose groups (Groups II, III, and IV) were similarly sectioned. (Note that all of the brains were processed and embedded at approximately the same time, even though the resulting paraffin blocks were sectioned at different time points.)

Morphometry

Mophometric measurements were initially performed in blinded fashion on sections from rats in each of the control and high dose (Group V) groups. Subsequently, similar measurements were performed in a non-blinded fashion on sections of brain from male and female rats in the remaining treatment groups. Twenty-one to 25 morphometric measurements were taken from each brain. (For the PND 22 rats, there were 21 measurements, with two more measurements being taken of the thickness of the corpus callosum than for the PND 10 brains. However, six measurements of the external germinal layer of the cerebellum were taken from the PND 10 rats. Only the mean value of these last six measurements is tabulated in the neuropathology report.) Two of the measurements were taken from the intact brains prior to trimming and processing, as elaborated above. The other measurements were taken from the histologic sections. Listed, below, are the microscopic morphometric measurements taken with a calibrated, ocular micrometer. (See also Figures 1 – 6).

1) Thickness of the frontal cortex. This measurement, taken bilaterally, was of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm and anterior commissure (Figures 1 an 4).
2) Thickness of the parietal cortex. This measurement, taken bilaterally, was of the dorsolateral portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm (Figures 1 and 4).
3) Diagonal (maximal) dimension, taken bilaterally, of the striatum and underlying globus pallidus, but not including the underlying nucleus accumbens (Figures 1 and 4).
4) Thickness of the corpus callosum, taken bilaterally, within the coronal section passing through the region of the optic chiasm (Figures 1 and 4). This measurement was taken at the level of the external granular layer of the overlying cingulate gyrus. For the PND 22 rats, two additional measurements were taken of the thickness of the corpus callosum (Figure 5). These were from a similar position relative to layer 2 of the overlying cortex but were taken within the section passing through the infundibulum.
5) Thickness of the hippocampal gyrus. This measurement, taken bilaterally, was performed on the dorsal portion of the hippocampus within the section taken at the level of the infundibulum (Figures 2 and 5).
6) Thickness of the dentate gyrus of the hippocampus. This measurement, taken bilaterally, was performed on the dorsal portion of the hippocampus within the section taken at the level of the infundibulum (Figures 2 and 5).
7) Thickness of the CA1 portion of the hippocampus. This measurement, taken bilaterally, was performed on the dorsal portion of the hippocampus within the section taken at the level of the infundibulum (Figures 2 and 5).

8) Thickness of the CA3 portion of the hippocampus. This measurement, taken bilaterally, was of the distance between the alveus and the dorsal blade of the dentate (Figures 2 and 5).
9) Maximum height of the cerebellum, usually at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface (Figures 3 and 6).
10) Thickness of the external germinal layer of the cerebellum (PND 10 rats only). Because the thickness of this layer varies considerably from region to region, six measurements were taken over the dorsum of the cerebellum and the mean value recorded as one measure (Figure 3).

All of the micrometric morphometric measurements were made by the study pathologist. An indelible ink drop was placed on each microslide adjacent to the measured section (*i.e.* corresponding to the most homologous section) and the measured sections digitally scanned using a PathScan Enabler™ and a Polaroid SprintScan 35® film scanner. These sections were scanned at a resolution of either 1350 or 2700 dpi, using a pre-scanning calibrated frame to standardize the image size. (Usually, these images are prepared at 2700 dpi, the maximum resolution of the scanner. However, the default setting was inadvertently changed to 1350 dpi for some of the scans and not detected immediately.) The digital images were saved in "TIFF" format, and these images then transferred to CD-R compact discs that were archived with the raw data file for this study. An attempt was made to specify the level of the first two measured sections according to A Stereotaxic Atlas of the Developing Rat Brain by Nancy M. Sherwood and Paola S. Timiras (Universty of California Press, 1970).[2] These level designations are also included with the histopathology raw data. The reader should note that this atlas is of Long Evans rats. Even though it includes coronal sections of brains of both PND 10 and PND 21 rats, many of the neuroanatomic features present at the measured levels did not match consistently with those of the Sprague Dawley rats used in this study. Furthermore, in the PND 10 rats, the appearance of the coronal sections taken at the same level (*e.g.* judged to be homologous by using a specific anatomic landmark such as the decussation of the anterior commissure as a reference point) varied considerably. Some structures were not measured due either to artifact (usually brain trauma induced during necropsy) or due to the fact that the sections were insufficiently homologous for comparison.

The statistical methods for the quantitative morphometric data were conducted by William Stiteler (Syracuse Research Corporation, Syracuse, N.Y.) and consisted of the following: 1) Bartlett's test for variance homogeneity (tested at the $p < 0.001$ level); 2) one-way analysis of variance (ANOVA)-parametric if Bartlett's test yielded a $p > 0.001$; 3) Dunnett's control versus treatment comparison t test if the ANOVA was significant at $p < 0.05$. The neuropathology reports for this study (one each for the PND 10 and PND 22 rats) should be consulted for these data.

RESULTS AND DISCUSSION

Brain weights and individual animal morphometric measurements may be found in the tables within the neuropathology reports on this study and are not repeated, here. The purpose of this report is primarily to present thumbnail images of the coronal brain sections that were measured. There are 78 pages of these thumbnail images appended to this introductory report. These thumbnails are not of sufficient magnification or resolution for critical study. However, larger-sized higher resolution digital images can be made available if needed for further evaluation. (These reside on six CD's, copies of which can be made.) The CD affixed to the back cover of this report also contains copies of each thumbnail page,

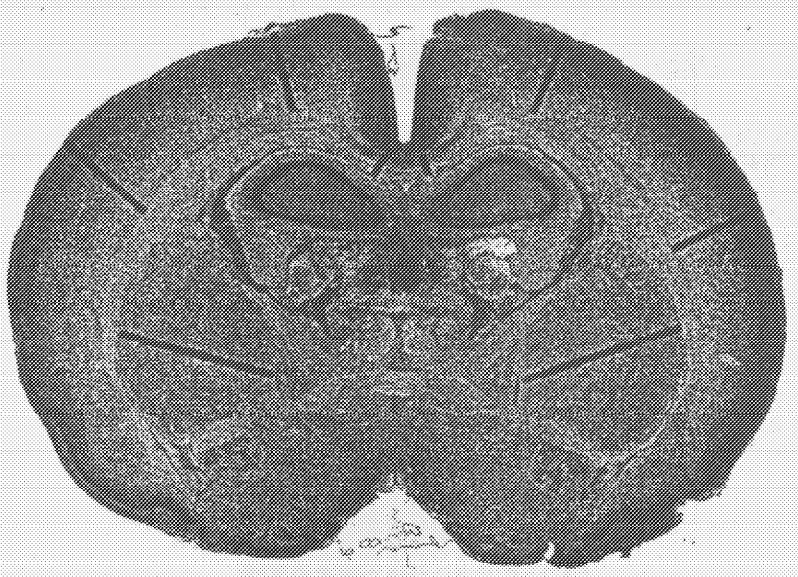


FIGURE 1 – Coronal section, level 1 (level of anterior commissure) of a PND 10 rat brain showing the measurements of the frontal cortex, parietal cortex, and striatum/globus pallidus. (H&E stain)

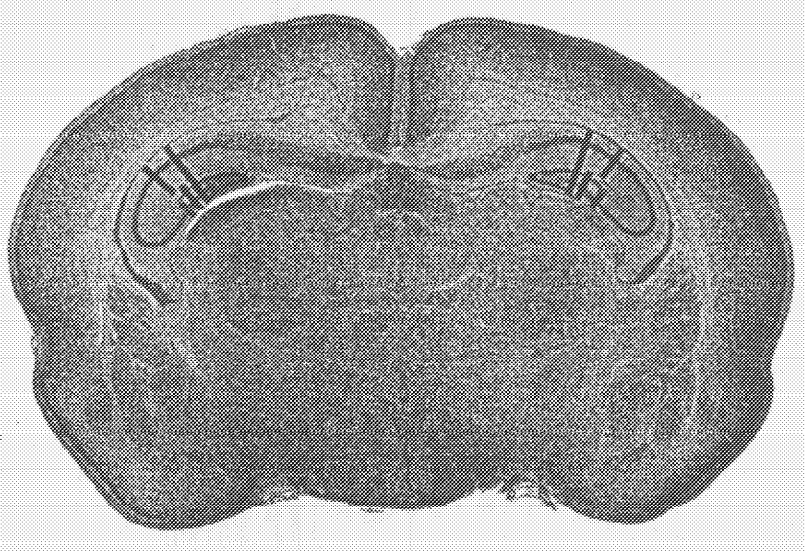


FIGURE 2 – Coronal section, level 2 (level of the infundibulum) of a PND 10 rat brain showing the four bilateral measurements taken of the hippocampus (full-hippocampal thickness, dentate, CA1, and CA3). Note the uneven nature of the corpus callosum in this section. The corpus callosum was often not present at this level in the PND 10 rat brains. (H&E stain)

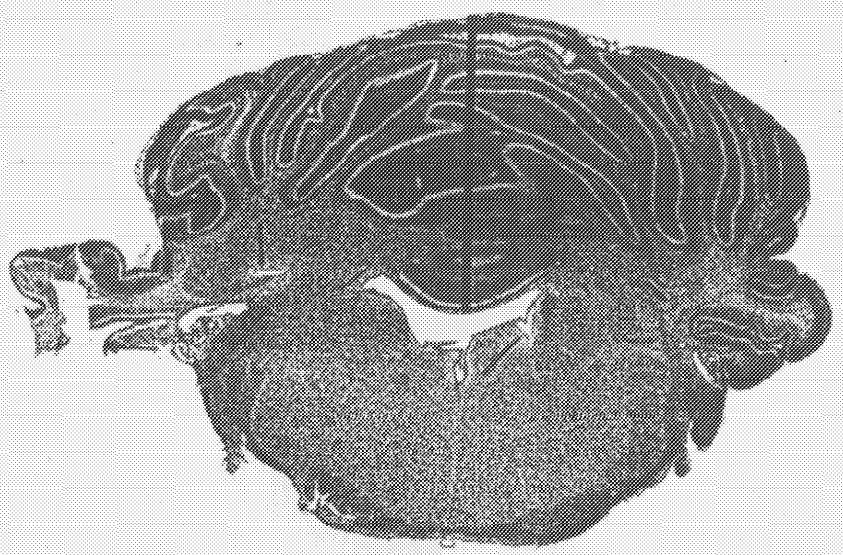


FIGURE 3 – Coronal section, level 4 (center of cerebellum) of a PND 10 rat showing the vertical full-thickness measurement that was taken. The external germinal layer is evident in this figure, but the specific locations of the six measurements taken of this layer are not depicted. The arrows point to foci of supependymal vesiculation and of vesiculation within the deep cerebellar nuclei. (H&E stain)

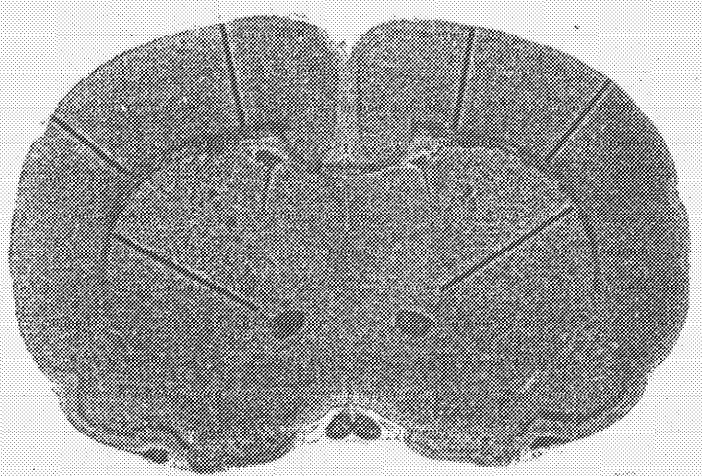


Figure 4 – Coronal section (level 1) taken at the level of the optic chiasm of a PND 22 rat showing the areas measured for the frontal cortex, parietal cortex, striatum, and corpus callosum. (LFB/CV stain)

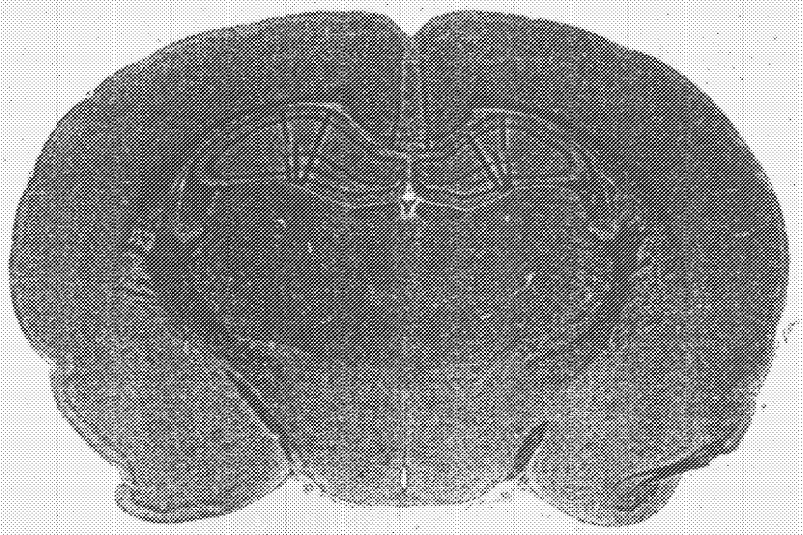


Figure 5 – Coronal section (level 2) taken at the level of the infundibulum of a PND 22 rat showing the regions measured for the hippocampus and corpus callosum. Note that the corpus callosum measurement does not include the dorsal hippocampal commissure. From medial to lateral, the hippocampal measures are of its full thickness, the dentate gyrus, the CA1 layer, and the CA3 layer. (LFB/CV stain)

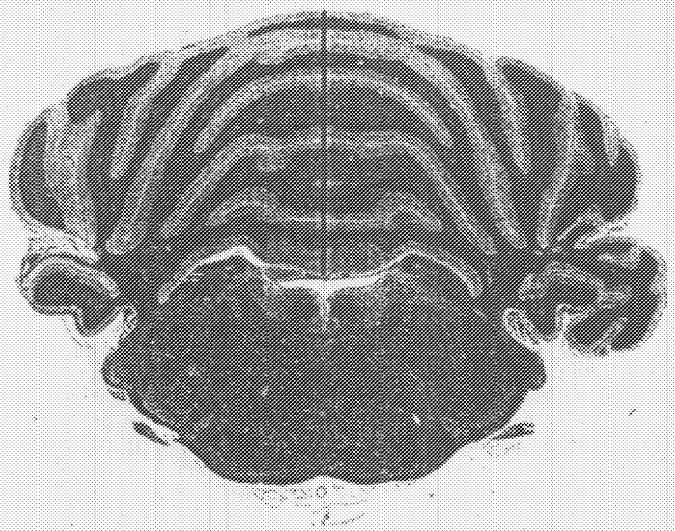


Figure 6 – Coronal section (level 4) taken just anterior to the middle of the cerebellum of a PND 22 rat. The superimposed line represents the full-thickness measurement of the cerebellar cortex. (LFB/CV stain)

and the images on this CD are in color. (Reminder: the PND 10 sections are stained with H&E, while the PND 22 sections are stained with LFB/CV.) The thumbnail images on the CD can be slightly enlarged without excessive pixilation developing, but they are of relatively low resolution. Furthermore, these thumbnail images were created in Adobe Photoshop 6.0®, and a compatible Photoshop program will undoubtedly be needed to view them.

There are three images for each rat, these being the sections on which measurements were taken. However, there were five brain block/rat and multiple sections on each slide. Several comments should be made about the sections depicted in the thumbnails. Firstly, a number of the sections from the PND 10 rats will be seen to be missing small portions (most frequently a portion of the dorsum of the cerebellum) or to be somewhat fragmented in appearance. These defects were most frequently the result of trauma to these soft brains induced during removal of the calvaria at the time of necropsy. Secondly, a much greater degree of variation will be seen in the appearance of the PND 10 sections than with the PND 22 sections. This is due to the much greater variability seen in both the size and degree of development of brains from rats at PND 10. (PND 10 corresponds to the time of major postnatal brain growth in the rat.) As previously mentioned, the microscopic appearances of the PND 10 brain sections often did not match the images present in the atlas authored by Sherwood and Timiras. For example, an attempt was made to have the anterior commissure within the Level 1 section. In this same section (*i.e.* in which the anterior commissure was present), the anterior portion of the dorsal hippocampus may or may not also have been present.

To briefly summarize the results of the morphometric evaluations performed on PND 10 brains, several trends were apparent. Firstly, all of the statistically significant differences for the treated male rats were for greater values in the rats exposed to the test chemical than for the controls. Secondly, the greatest numbers of affected areas and greatest statistical significances were present in the Group IV postpartum day 10 male rats. Thirdly, there was a reverse trend in the female rats – *i.e.* for decreases in certain dimensions in test substance-treated groups. The neuroanatomic regions that most consistently showed statistically significant inter-group differences were the corpus callosum for the male rats and the CA1 region of the hippocampus for the females. For the PND 10 rats, the corpus callosa were found to be significantly thicker for the male rats in Groups III and IV in comparison to the Control Group values. The mean values for the thickness of the corpus callosa were also higher in Group V than in the Control group, but this inter-group difference was not statistically significant. For the female rats, the mean thicknesses of the corpus callosa were thinner in treated groups than in the Control Group, but these differences were not statistically significant. Note that the corpus callosa are poorly developed at the PND 10 stage, so measurements of thickness could only be performed consistently within the Level 1 section.

For the PND 22 rats, measurements of the thickness of the corpus callosum were made at two levels (see Figures 4 and 5). The corpus callosa of both the male and female PND 22 rats were slightly thinner in the more anterior section (*i.e.* level of the anterior commissure) than in the comparable control rats, while the more posterior region (level of the infundibulum) was thicker than in the controls. It is worth emphasizing that the posterior measurement of the corpus callosum within the PND 22 rat brains was taken quite close to or within the region of the splenium, a location in which the corpus callosum normally becomes thicker. Therefore, it is possible that the test substance exposure may have resulted in shortening of the anterior to posterior length of this white matter tract (as apposed

to causing it to become thicker by some other mechanism such as a decrease in axon loss during the "dying back" period of development). Sagittal sections could be prepared from some of the brains of the PND 5 rats present on this study in order to check for such a possibility.

Other than for the bimodal pattern of corpus callosum thickening in the PND 22 rat brains, the pattern of inter-group difference seen in the morphometric parameters for the PND 22 rats was similar to that encountered with the PND 10 rats present on this study. These data suggested not only a treatment-related effect on brain development but that there may also have been some degree of sexual dimorphism regarding response of rats to the test substance.

CONCLUSIONS

The presence of some apparent treatment-related differences in simple linear measurements has created the need for additional expert input from developmental neurobiologists *re:* both the validity of the morphometry data and the biologic significance of the same. In order to assist in any efforts by the consultants to ascertain the degree of homology between sections, thumbnail images of the measured sections are presented as an appendix to this report. CD's bearing the individual brain scans may also be created for evaluation by the consultants. Additional studies may be indicated to either substantiate or refute the results of this initial morphometric evaluation. Area measurements could be performed on selected neuroanatomic structures. In addition, sagittal brain sections could be prepared either from the brains of the PND 5 rats on this study or from rats on an additional study in order to measure the anterior to posterior length of the corpus callosa or to do evaluations of densities of crossing fiber tracts (much easier to do on cross sections of axons than on longitudinal sections). The degree to which the brain sizes might reflect a treatment-related nutritional difference (*e.g via* differences in composition of the mother's milk) should naturally be explored but does not fit as well with the sexually dimorphic pattern present in this study as would a hormonal mechanism.

2-22-01

Robert H. Garman, DVM — Date
Diplomate, ACVP

THUMBNAIL IMAGE APPENDIX BACKGROUND INFORMATION

Comments: There are 78 pages of thumbnail images. There are three images/rat and four rats/page. All of the PND 10 brain sections were scanned at the same magnification. The same applies for the PND 22 rats, but the magnifications for the PND 10 and PND 22 rats are different. For the PND 10 rats, the image-grabbing frame was set at 7.5 x 10 mm. For the PND 22 rats, this frame was set at 10 x 15 mm.

These thumbnail images were created in Adobe Photoshop 6.0®. Although similar images (in color) are also present on the CD affixed to the back cover of this report, the images on this CD may not be readable by a non-Photoshop® image program.

Each image has the individual animal number below it followed by the sex designation (M or F) and then the slide/block number (#1, #2, or #4). Slide #3 bears sections of the midbrain, and no measurements were performed on these sections. Slide #5 bears sections of all of the remaining brain slices. Although the treatment group numbers could not be added directly to these thumbnail proof sheets, the treatment groups are in numerical order, with the Group I rats being presented first and the Group V animals being last. The individual animal numbers by treatment group are as follows:

PND 10 Rats (Note that the starting number is slightly different for the Group III males and females.

Group I: 16616 to 16637
Group II: 16640 to 16659
Group III: 16662 to 16683
Group IV: 16685 to 16707
Group V: 16708 to 16730

PND 22 Rats

Group I: 16731 to 16753
Group II: 16755 to 16776
Group III: 16781 to 16799
Group IV: 16800 to 16822
Group V: 16823 to 16844

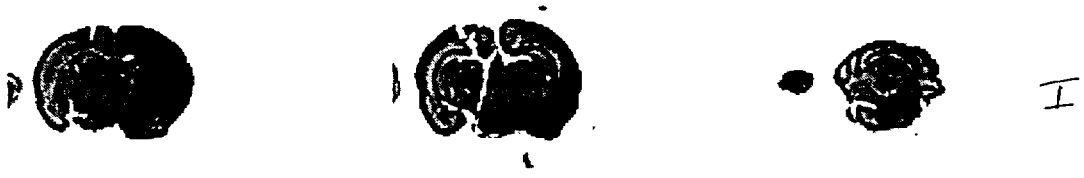
"THUMBNAIL" IMAGES OF SCANNED BRAIN SECTIONS

PROTOCOL 1416-003

Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or *via* Maternal Milk

Scanned Sections from F_1 Generation Day 10 Postpartum Male Rats

Study 1416-003 – Contact Sheets for Day 10 Male Rats



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16618M-1 16618M-2 16618M-4 I

16619M-1 16619M-2 16619M-4 I

Study 1416-003 – Contact Sheets for Day 10 Male Rats

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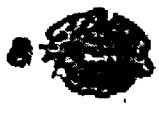
16620M-2



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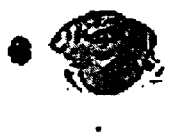

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Study 1416-003 – Contact Sheets for Day 10 Male Rats

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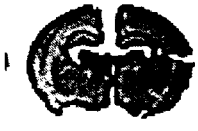

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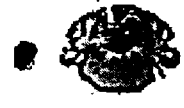

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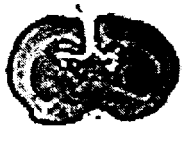

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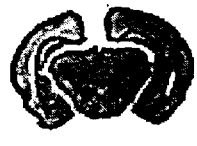

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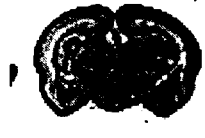

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Study 1416-003 – Contact Sheets for Day 10 Male Rats


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Study 1416-003 – Contact Sheets for Day 10 Male Rats

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Study 1416-003 – Contact Sheets for Day 10 Male Rats

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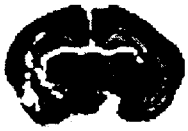

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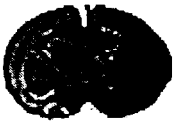
16669M-4

Study 1416-003 – Contact Sheets for Day 10 Male Rats



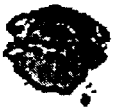
16670M-1

16670M-2

16670M-4

16673M-1

16673M-2

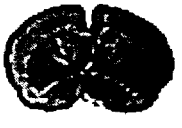


16673M-4

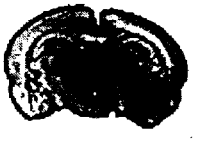
16675M-1

16675M-2

16675M-4



16676M-1



16676M-2

16676M-4

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16678M-1

16678M-2

16678M-4

16679M-1

16679M-2

16679M-4

16683M-1

16683M-2

16683M-4

16685M-1

16685M-2

16685M-4

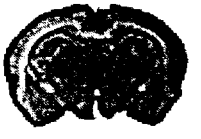
Study 1416-003 – Contact Sheets for Day 10 Male Rats

16686M-1

16686M-2

16686M-4 IV

16687M-1



16687M-2

16687M-4 IV

16688M-1

16688M-2

16688M-4 IV

16689M-1

16689M-2

16689M-4 IV

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16690M-1

16690M-2

16690M-4 IV

16692M-1

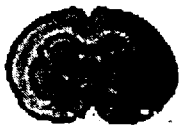
16692M-2

16692M-4 IV

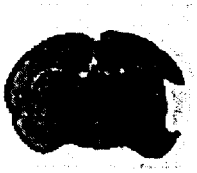
16693M-1

16693M-2

16693M-4 IV



16695M-1



16695M-2

16695M-4 IV

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16696M-1

16696M-2

IV

16696M-4

16697M-1

16697M-2

IV

16697M-4

16698M-1

16698M-2

IV

16698M-4

16699M-1

16699M-2

IV

16699M-4

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16701M-1

16701M-2

16701M-4

16706M-1

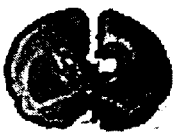
16706M-2

16706M-4

16707M-1

16707M-2

16707M-4



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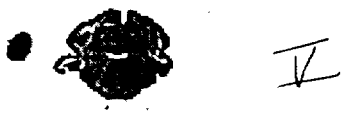
16708M-2

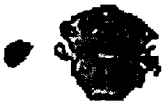


16708M-4

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16709M-1

16709M-2



16709M-4

16711M-1

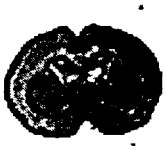
16711M-2

16711M-4

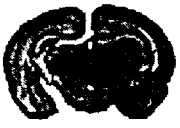
16712M-1

16712M-2

16712M-4



16713M-1



16713M-2

16713M-4

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16715M-1

16715M-2

IV

16715M-4

16717M-1

16717M-2

IV

16717M-4

16718M-1

16718M-2

IV

16718M-4

16719M-1

16719M-2

IV

16719M-4

Study 1416-003 – Contact Sheets for Day 10 Male Rats

16722M-1

16722M-2

16722M-4 IV

16723M-1

16723M-2

16723M-4 IV

16724M-1

16724M-2

16724M-4 IV

16725M-1

16725M-2

16725M-4 IV

Study 1416-003 – Contact Sheets for Day 10 Male Rats

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16728M-2

16728M-4

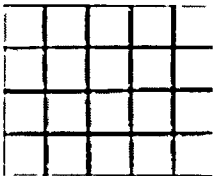
V

16730M-1

16730M-2

16730M-4

V



Frame

"THUMBNAIL" IMAGES OF SCANNED BRAIN SECTIONS

PROTOCOL 1416-003

Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or *via* Maternal Milk

Scanned Sections from F_1 Generation Day 10 Postpartum Female Rats

Study 1416-003 – Contact Sheets for Day 10 Female Rats


16616F-1


16616F-2


I

16616F-4


16617F-1


16617F-2


I

16617F-4


16618F-1


16618F-2


I

16618F-4


16619F-1


16619F-2


I

16619F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

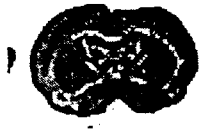
16620F-1 16620F-2 16620F-4 I

16621F-1 16621F-2 16621F-4 I

16623F-1 16623F-2 16623F-4 I

16625F-1 16625F-2 16625F-4 I

Study 1416-003 – Contact Sheets for Day 10 Female Rats


16626F-1


16626F-2


16626F-4 I


16629F-1

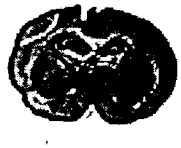

16629F-2


16629F-4 I

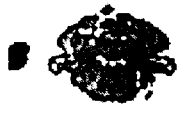

16630F-1


16630F-2


16630F-4 I


16631F-1


16631F-2


16631F-4 I

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16634F-1

16634F-2

16634F-4 I

16635F-1

16635F-2

16635F-4 I

16636F-1

16636F-2

16636F-4 I

16637F-1

16637F-2

16637F-4 I

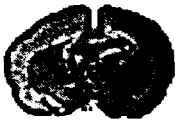
Study 1416-003 – Contact Sheets for Day 10 Female Rats

16640F-1

16640F-2

II

16640F-4



16641F-1

16641F-2

II

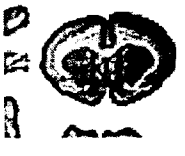
16641F-4

16643F-1

16643F-2

II

16643F-4



16644F-1

16644F-2

II

16644F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16645F-1

16645F-2

II

16645F-4

16646F-1

16646F-2

II

16646F-4

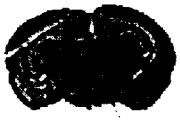
16647F-1

16647F-2

II

16647F-4

16649F-1



16649F-2

II

16649F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16650F-1

16650F-2

16650F-4

II

16651F-1

16651F-2

16651F-4

II

16653F-1

16653F-2

16653F-4

II

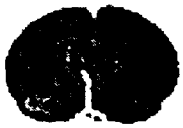
16654F-1

16654F-2

16654F-4

II

Study 1416-003 – Contact Sheets for Day 10 Female Rats



16655F-1

16655F-2

II

16655F-4

16656F-1

16656F-2

II

16656F-4

16657F-1

16657F-2

II

16657F-4

16659F-1

16659F-2

II

16659F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16662F-1

16662F-2

16662F-4

16664F-1

16664F-2

16664F-4

16665F-1

16665F-2

16665F-4

16667F-1

16667F-2

16667F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16668F-1

16668F-2

16668F-4

III

16669F-1

16669F-2

16669F-4

III

16670F-1

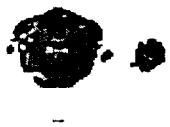
16670F-2

16670F-4

III

16673F-1

16673F-2



16673F-4

III

Study 1416-003 – Contact Sheets for Day 10 Female Rats



16675F-1 16675F-2 16675F-4



16676F-1 16676F-2 16676F-4



16678F-1 16678F-2 16678F-4

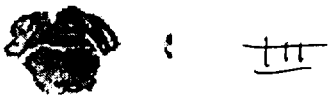


16679F-1 16679F-2 16679F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16680F-1

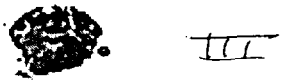
16680F-2



16680F-4

16681F-1

16681F-2



16681F-4

16683F-1

16683F-2

16683F-4

16685F-1

16685F-2

16685F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16686F-1

16686F-2

IV

16686F-4

16688F-1

16688F-2

IV

16688F-4

16689F-1

16689F-2

IV

16689F-4

16690F-1

16690F-2

IV

16690F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16692F-1

16692F-2

16692F-4 IV

16693F-1

16693F-2

16693F-4 IV

16695F-1

16695F-2

16695F-4 IV

16696F-1

16696F-2

16696F-4 IV

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16697F-1

16697F-2

16697F-4 IV

16698F-1

16698F-2

16698F-4 IV

16699F-1

16699F-2

16699F-4 IV

16701F-1

16701F-2

16701F-4 IV

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16706F-1

16706F-2

16706F-4 IV

16707F-1

16707F-2

16707F-4 IV

16708F-1

16708F-2

16708F-4 V

16709F-1

16709F-2

16709F-4 V

Study 1416-003 – Contact Sheets for Day 10 Female Rats

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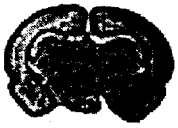
16712F-1

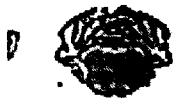
16712F-2

16712F-4

16713F-1



16713F-2



16713F-4

16715F-1

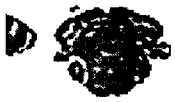
16715F-2

16715F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16716F-1

16716F-2



16716F-4

16717F-1

16717F-2

16717F-4

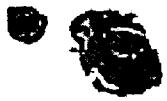
16718F-1

16718F-2

16718F-4

16719F-1

16719F-2



16719F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16723F-1

16723F-2

V

16723F-4

16724F-1

16724F-2

V

16724F-4

16725F-1

16725F-2

V

16725F-4

16728F-1

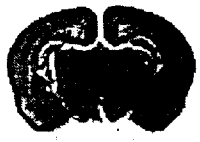
16728F-2

V

16728F-4

Study 1416-003 – Contact Sheets for Day 10 Female Rats

16730F-1



16730F-2

16730F-4

IV

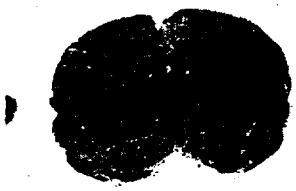
"THUMBNAIL" IMAGES OF SCANNED BRAIN SECTIONS

PROTOCOL 1416-003

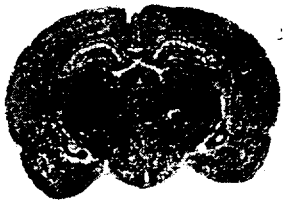
Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or *via* Maternal Milk

Scanned Sections from F_1 Generation Day 22 Postpartum Male Rats

Study 1416-003 – Contact Sheets for Day 22 Male Rats



16731M-1

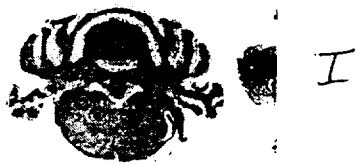


16731M-2

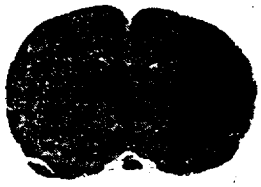
16731M-4

16732M-1

16732M-2



16732M-4

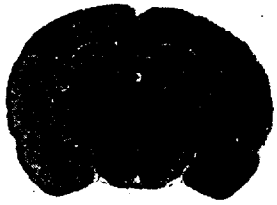


16734M-1

16734M-2

16734M-4

16735M-1

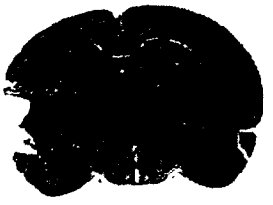


16735M-2

16735M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

16736M-1



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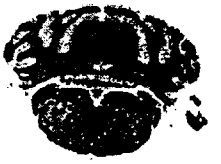
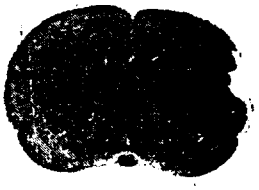
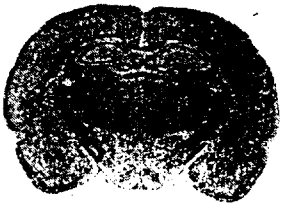


I

16736M-4



16737M-1



16737M-2

I

16737M-4

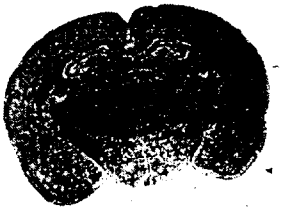
16738M-1

16738M-2

I

16738M-4

16740M-1

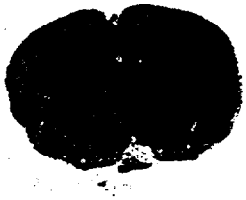


16740M-2

I

16740M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

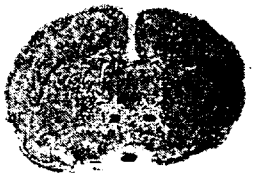


16741M-1

16741M-2

I

16741M-4



16742M-1

16742M-2

I

16742M-4

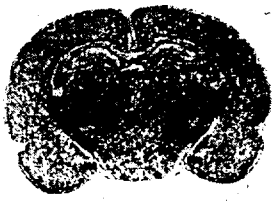
16745M-1

16745M-2

I

16745M-4

16746M-1

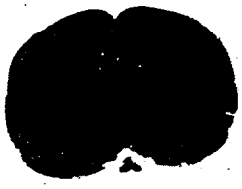


16746M-2

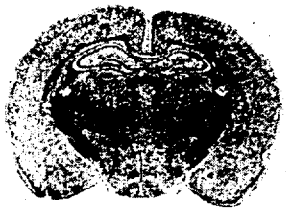
I

16746M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats



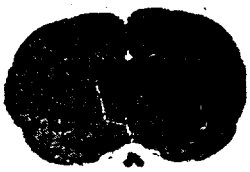
16747M-1



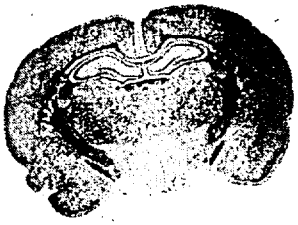
16747M-2

I

16747M-4



16749M-1



16749M-2

I

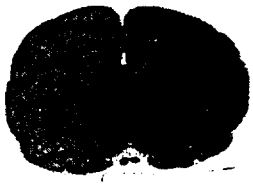
16749M-4

16750M-1

16750M-2

I

16750M-4



16753M-1

16753M-2

I

16753M-4

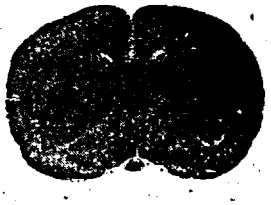
Study 1416-003 – Contact Sheets for Day 22 Male Rats

16755M-1

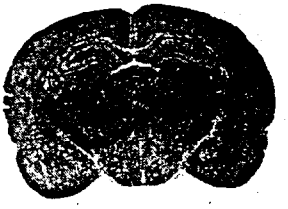
16755M-2

II

16755M-4



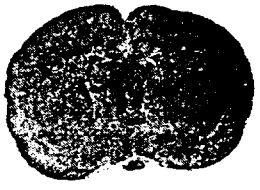
16758M-1



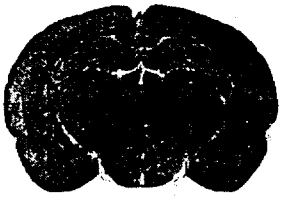
16758M-2

II

16758M-4



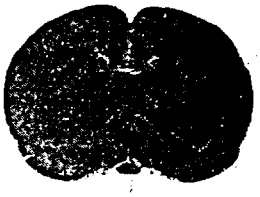
16760M-1



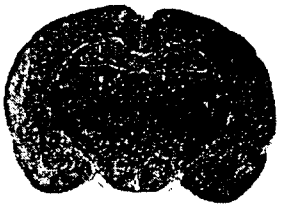
16760M-2

II

16760M-4



16761M-1



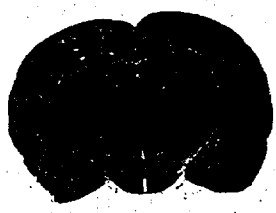
16761M-2

II

16761M-4

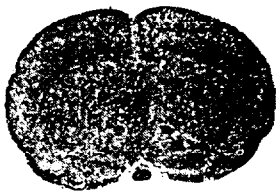
Study 1416-003 – Contact Sheets for Day 22 Male Rats

16762M-1

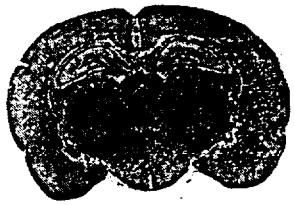


16762M-2

16762M-4

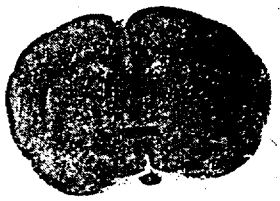


16763M-1



16763M-2

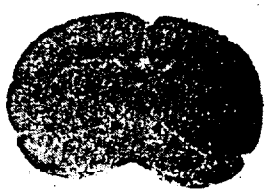
16763M-4



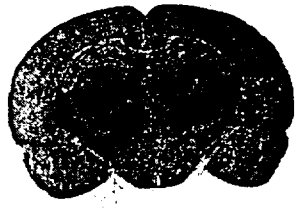
16764M-1

16764M-2

16764M-4



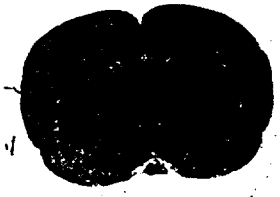
16765M-1



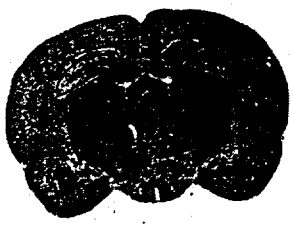
16765M-2

16765M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

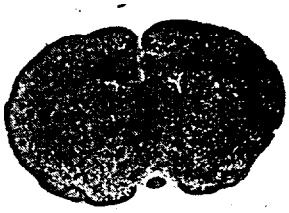


16767M-1

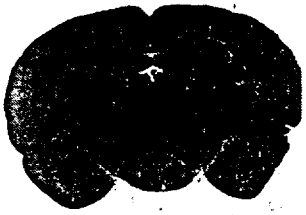


16767M-2

16767M-4

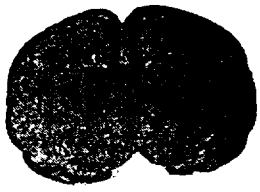


16768M-1

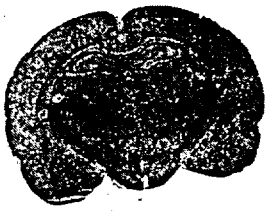


16768M-2

16768M-4

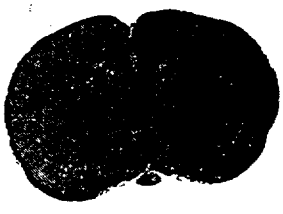


16769M-1



16769M-2

16769M-4



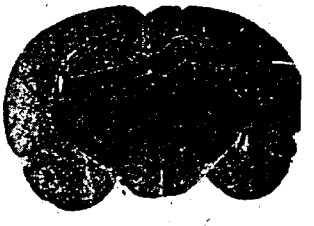
16770M-1

16770M-2

16770M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

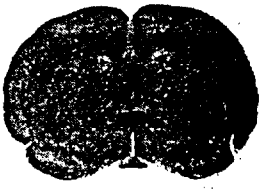
16771M-1



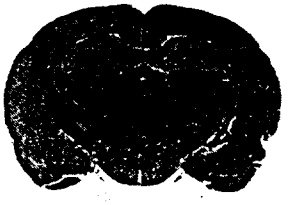
16771M-2

II

16771M-4



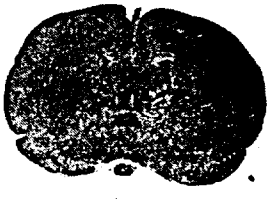
16773M-1



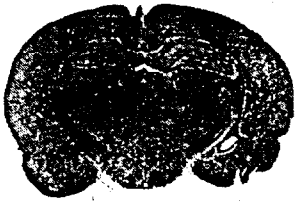
16773M-2

II

16773M-4



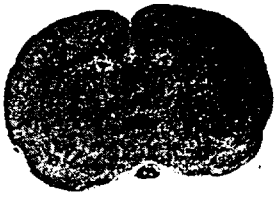
16774M-1



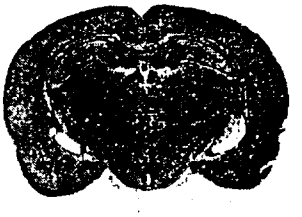
16774M-2

II

16774M-4



16776M-1

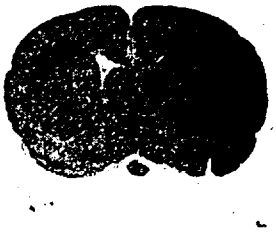


16776M-2

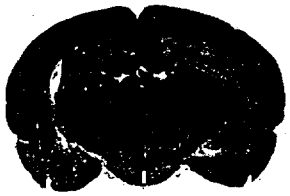
II

16776M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

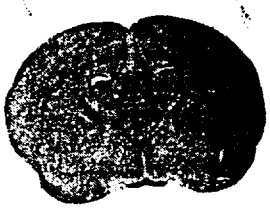


16781M-1

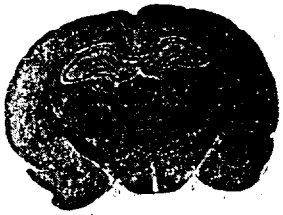


16781M-2

16781M-4



16782M-1



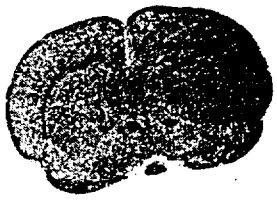
16782M-2

16782M-4

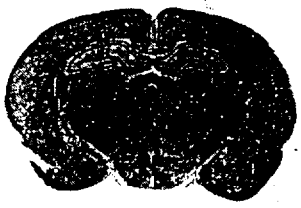
16783M-1

16783M-2

16783M-4



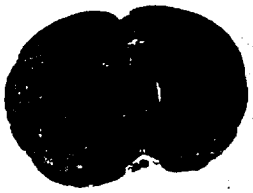
16784M-1



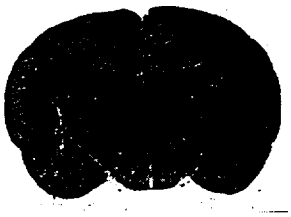
16784M-2

16784M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

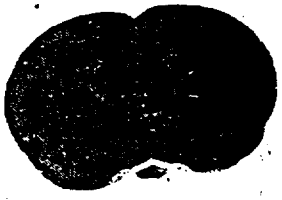


16786M-1

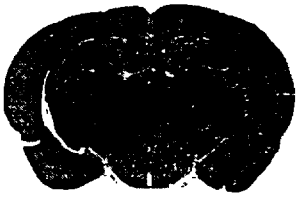


16786M-2

16786M-4

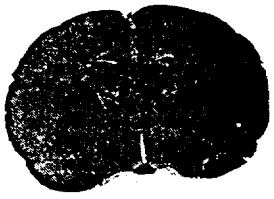


16787M-1



16787M-2

16787M-4

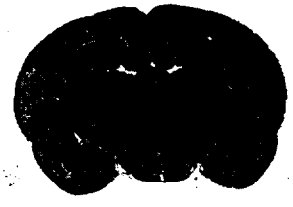


16791M-1

16791M-2

16791M-4

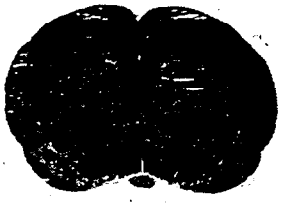
16792M-1



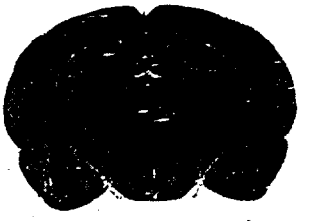
16792M-2

16792M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats



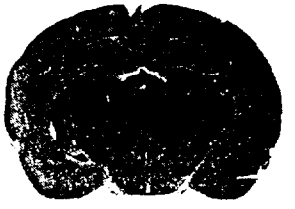
16793M-1



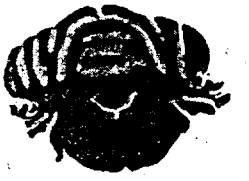
16793M-2

16793M-4

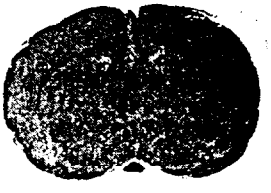
16794M-1



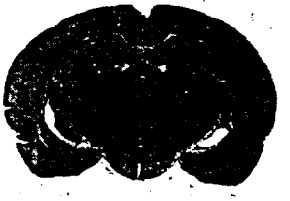
16794M-2



16794M-4



16795M-1



16795M-2

16795M-4

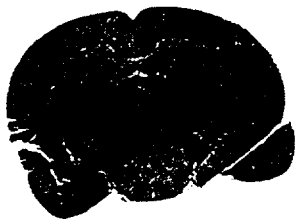
16796M-1

16796M-2

16796M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

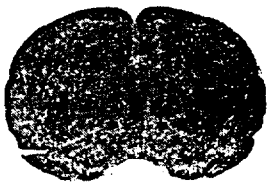
16797M-1



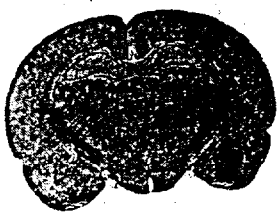
16797M-2

III

16797M-4



16798M-1

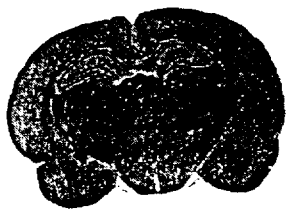


16798M-2

III

16798M-4

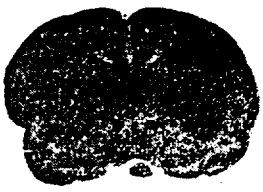
16799M-1



16799M-2

III

16799M-4



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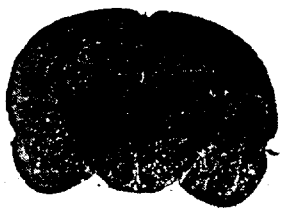
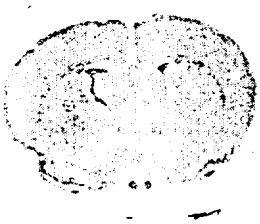


16800M-2

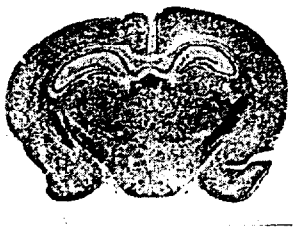
III
IV

16800M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats



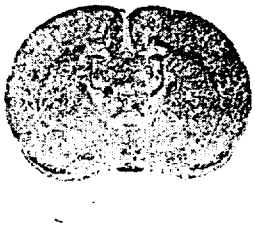
16801M-1



16801M-2

IV

16801M-4

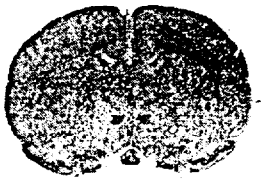


16802M-1

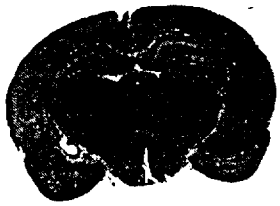
16802M-2

IV

16802M-4



16803M-1

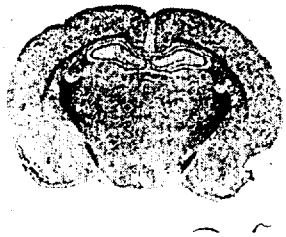


16803M-2

IV

16803M-4

16805M-1

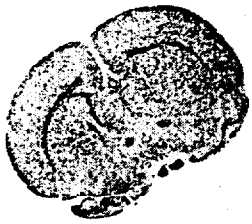


16805M-2

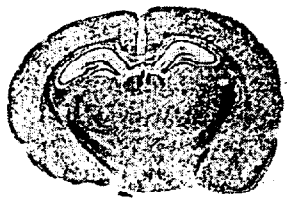
IV

16805M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

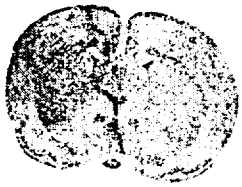


16806M-1



16806M-2

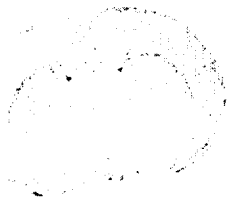
16806M-4



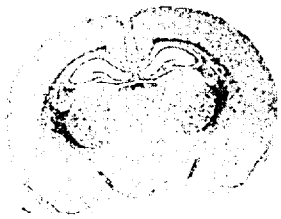
16807M-1

16807M-2

16807M-4

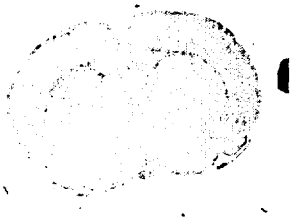


16808M-1

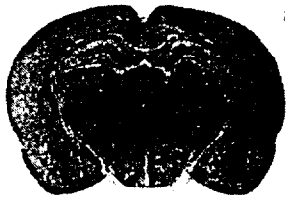


16808M-2

16808M-4



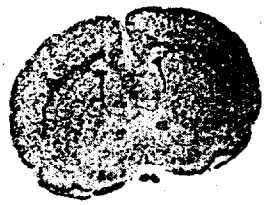
16810M-1



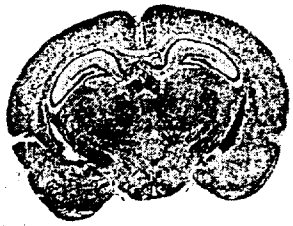
16810M-2

16810M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats



16811M-1



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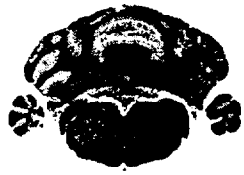
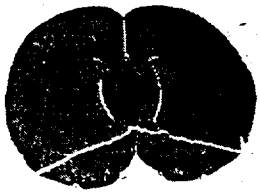
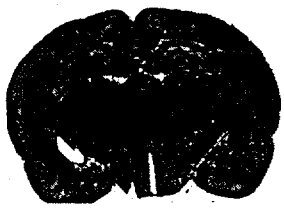


16811M-4



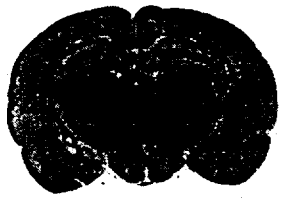
16814M-1



16814M-2

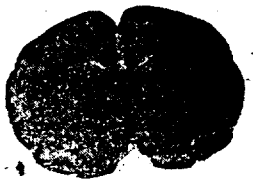
16814M-4

16815M-1



16815M-2

16815M-4



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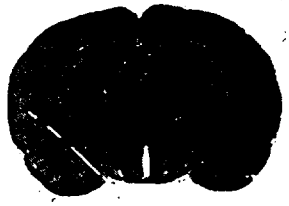
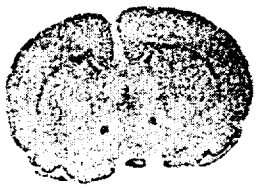


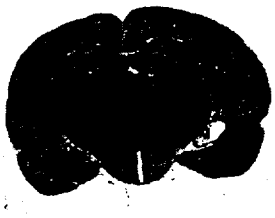
16817M-2

16817M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats



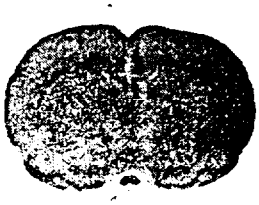
16818M-1



16818M-2

IV

16818M-4

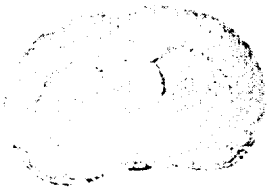


16820M-1

16820M-2

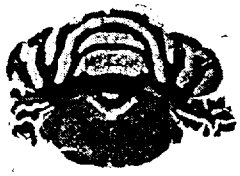
IV

16820M-4



16822M-1

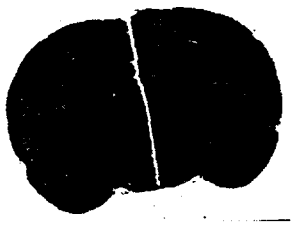
16822M-2



IV

16822M-4

16823M-1



16823M-2

V

16823M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

16825M-1

16825M-2

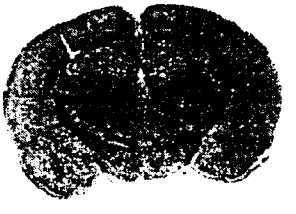
16825M-4

16826M-1

16826M-2

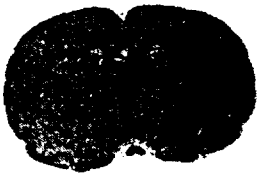
16826M-4

16827M-1

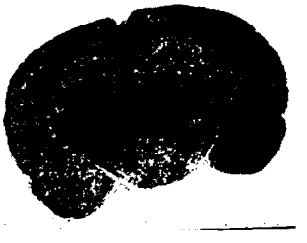


16827M-2

16827M-4



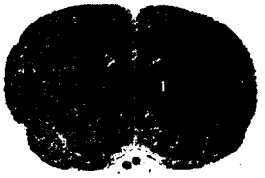
16828M-1



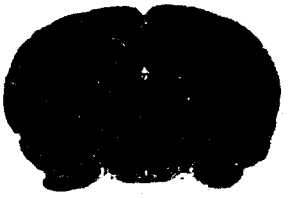
16828M-2

16828M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

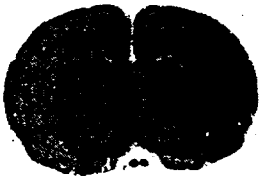


16830M-1



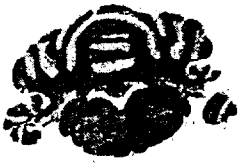
16830M-2

16830M-4



16831M-1

16831M-2



16831M-4

16833M-1

16833M-2

16833M-4

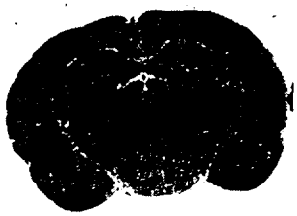
16834M-1

16834M-2

16834M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

16836M-1



16836M-2

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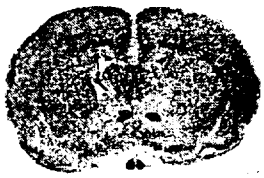
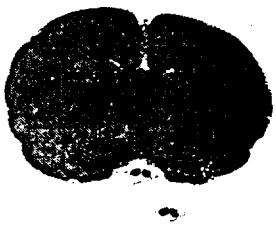
16836M-4



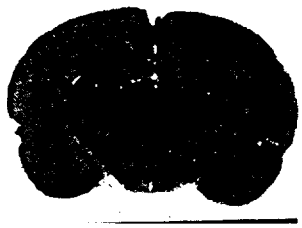
16837M-1

16837M-2

16837M-4

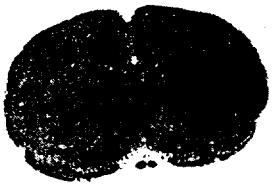


16839M-1

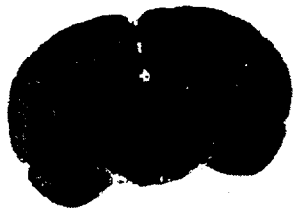


16839M-2

16839M-4



16840M-1



16840M-2

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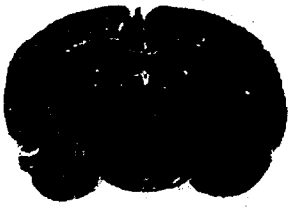
16840M-4

Study 1416-003 – Contact Sheets for Day 22 Male Rats

16842M-1

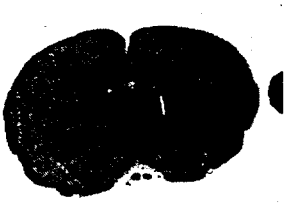


16842M-2

[redacted]

V

16842M-4



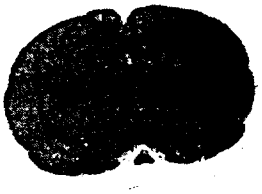
16843M-1

16843M-2

[redacted]

V

16843M-4



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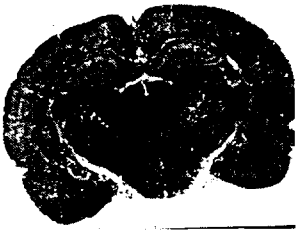
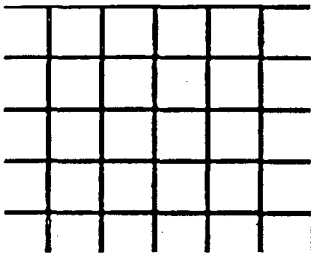


16844M-2

V

16844M-4



GRID

"THUMBNAIL" IMAGES OF SCANNED BRAIN SECTIONS

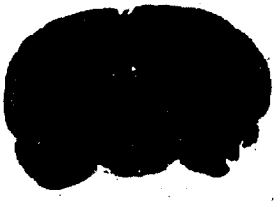
PROTOCOL 1416-003

Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or *via* Maternal Milk

Scanned Sections from F_1 Generation Day 22 Postpartum Female Rats

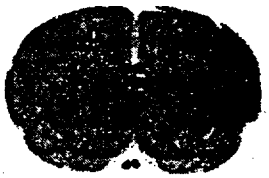
Study 1416-003 – Contact Sheets for Day 22 Female Rats

16731F-1

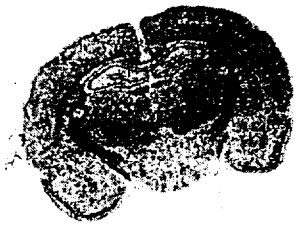


16731F-2

16731F-4



16732F-1



16732F-2

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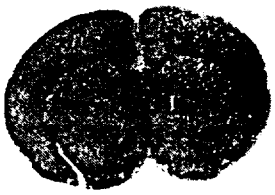
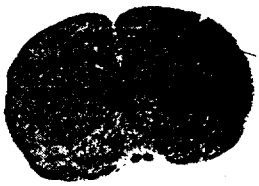
16732F-4



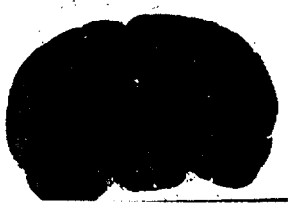
16734F-1

16734F-2

16734F-4



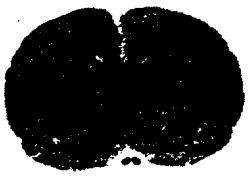
16735F-1



16735F-2

16735F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



16736F-1

16736F-2

I

16736F-4

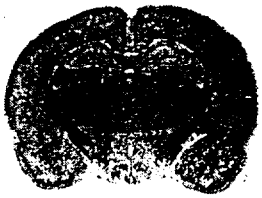
16737F-1

16737F-2

I

16737F-4

16738F-1



16738F-2

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16738F-4

16740F-1

16740F-2

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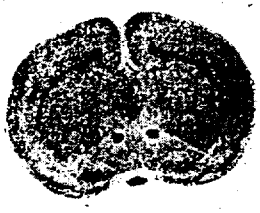
16740F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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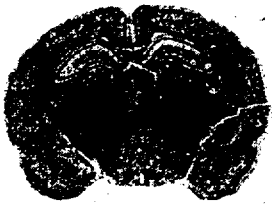

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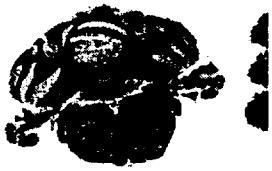

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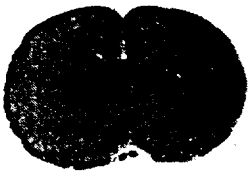
16741F-4

16742F-1

16742F-2

I

16742F-4



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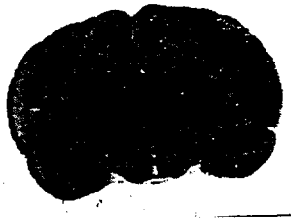

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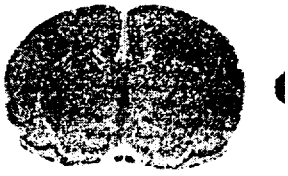
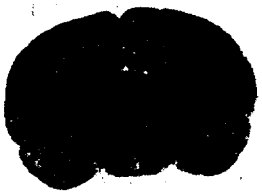
16745F-4



16746F-1



16746F-2

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16746F-4

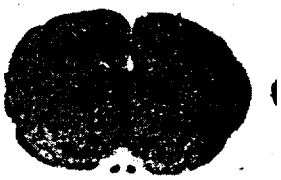
Study 1416-003 – Contact Sheets for Day 22 Female Rats

16747F-1

16747F-2

I

16747F-4



16749F-1

16749F-2

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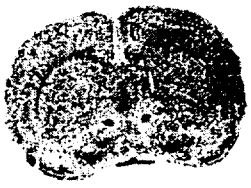
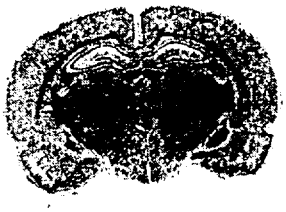
16749F-4



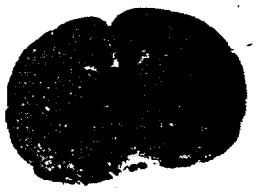
16750F-1



16750F-2

I

16750F-4



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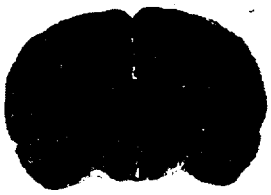
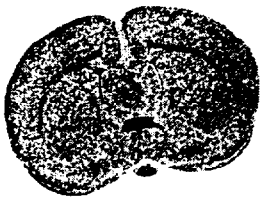


16753F-2

I

16753F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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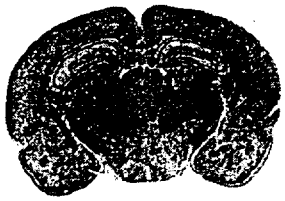
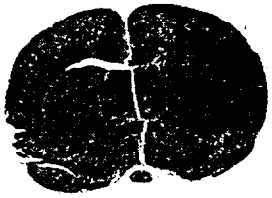


16755F-2

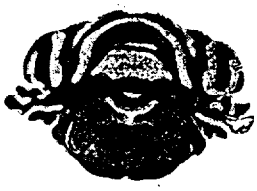
II

16755F-4



16758F-1

16758F-2



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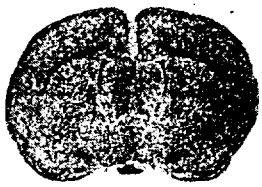
16758F-4



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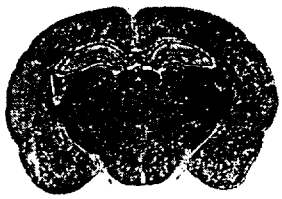
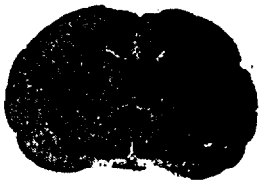


16760F-2

II

16760F-4



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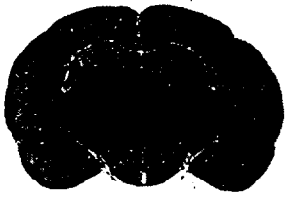
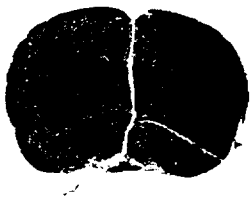


16761F-2

II

16761F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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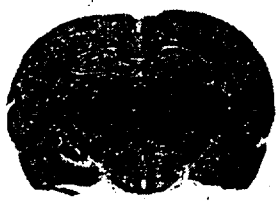

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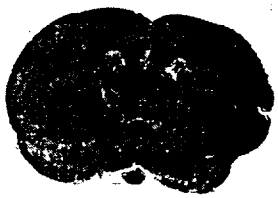
16762F-4



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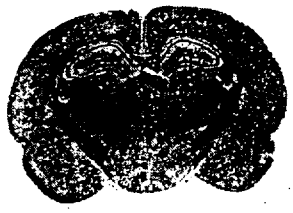

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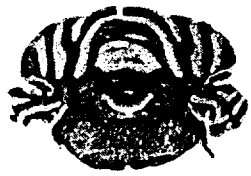

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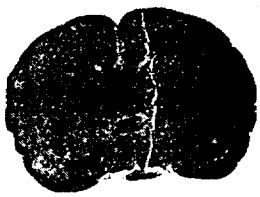

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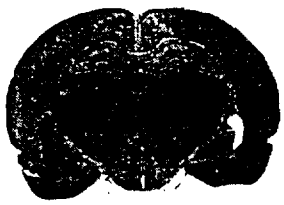

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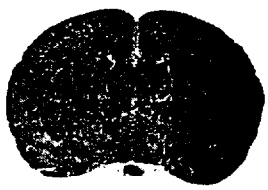
16764F-4



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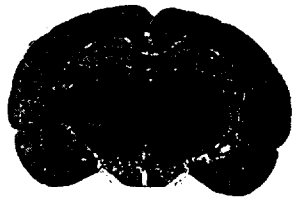
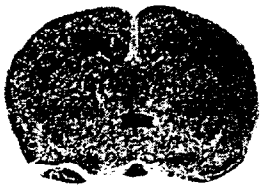


16765F-2

16765F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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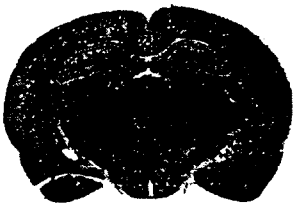
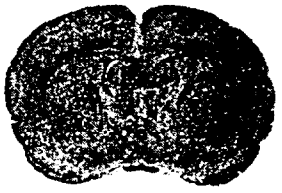


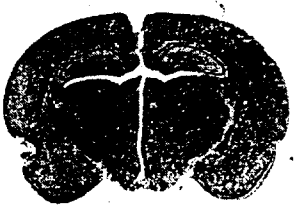
16767F-2

II

16767F-4



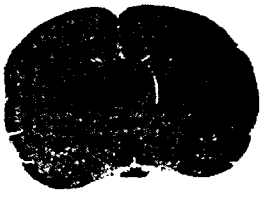
16768F-1



16768F-2

II

16768F-4



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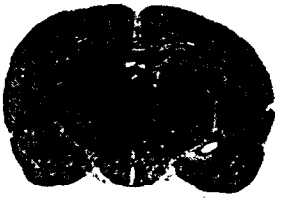
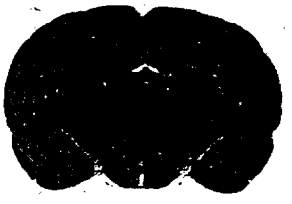


16769F-2

II

16769F-4

16770F-1

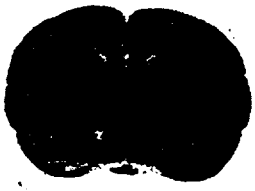


16770F-2

II

16770F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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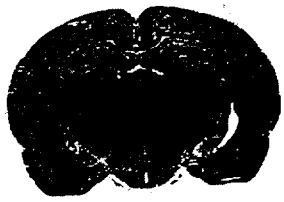

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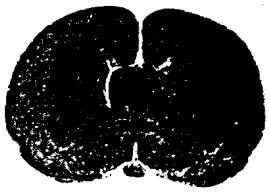
16771F-4



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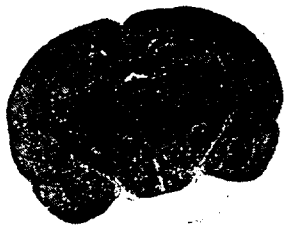

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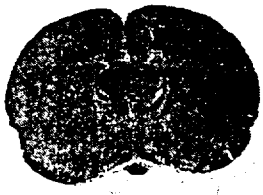

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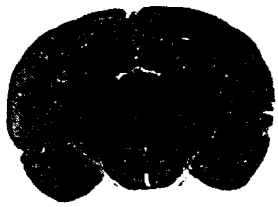

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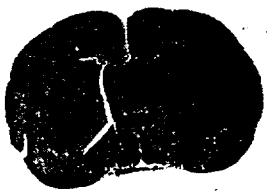

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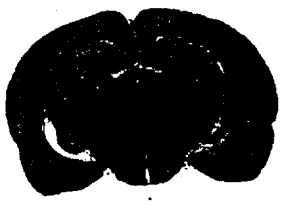

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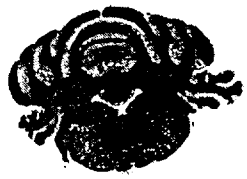

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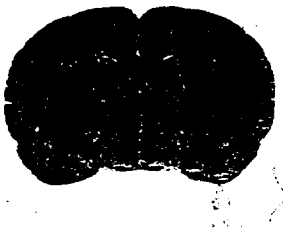
Study 1416-003 – Contact Sheets for Day 22 Female Rats

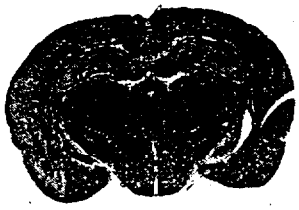


16781F-1

16781F-2

16781F-4

16782F-1



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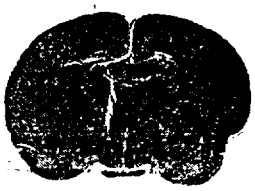

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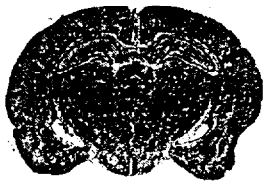

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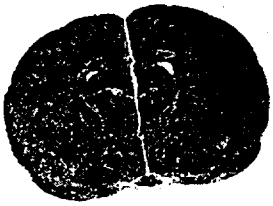

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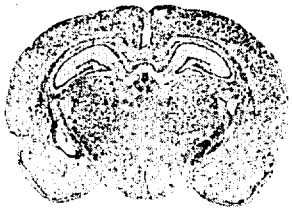

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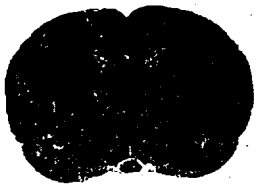
Study 1416-003 – Contact Sheets for Day 22 Female Rats



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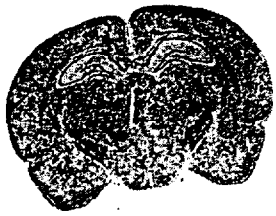

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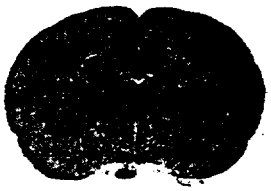

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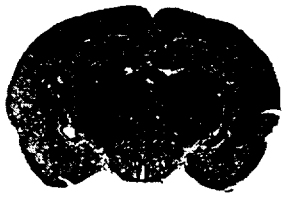

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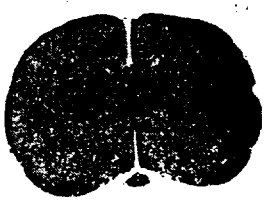

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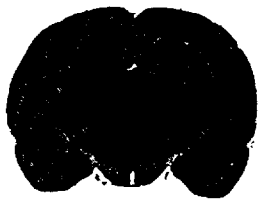

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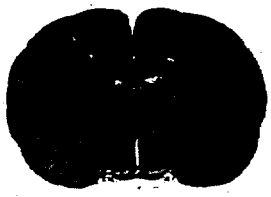
Study 1416-003 – Contact Sheets for Day 22 Female Rats



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16794F-1

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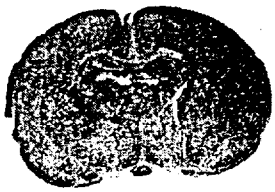

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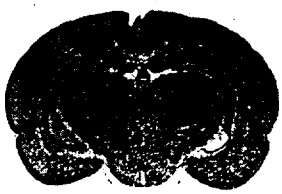

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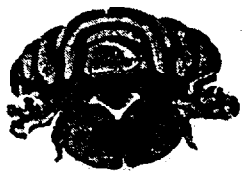

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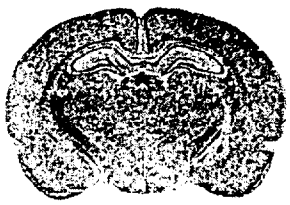

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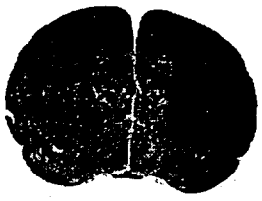
16796F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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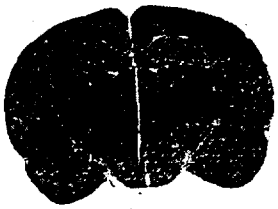

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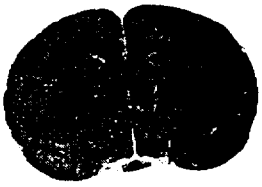

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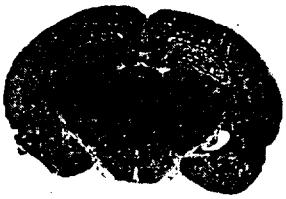

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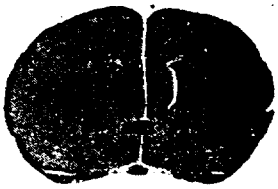

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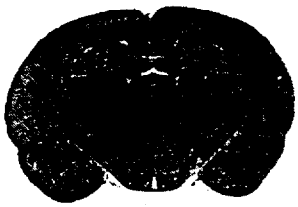

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III

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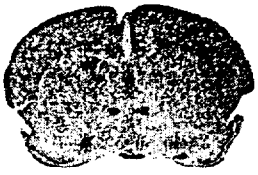

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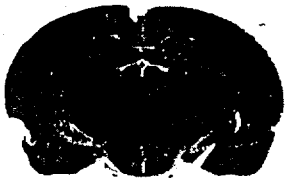

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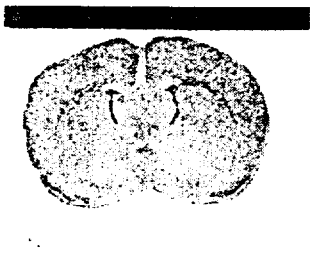
IV

16800F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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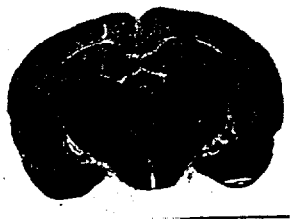

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IV

16801F-4

16802F-1

16802F-2

IV

16802F-4

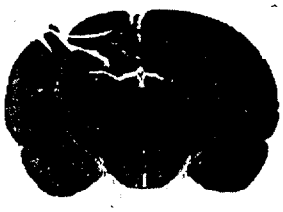
16803F-1

16803F-2

IV

16803F-4

16805F-1



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16805F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats

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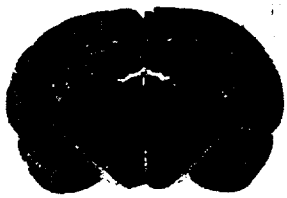

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IV

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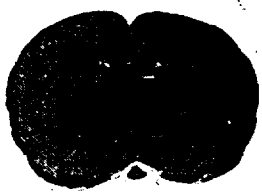

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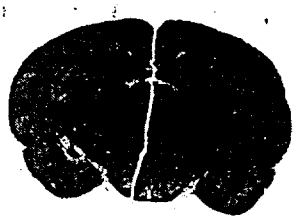

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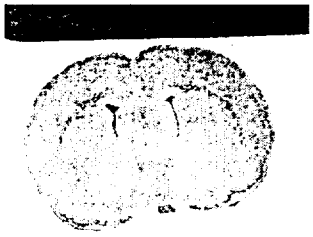
16807F-4



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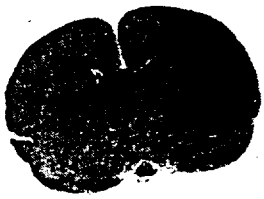

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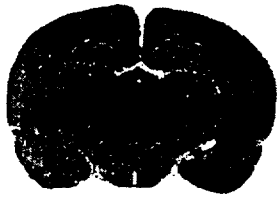

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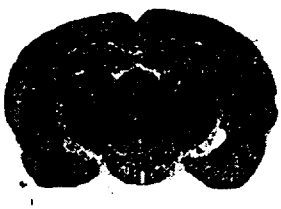
IV

16810F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats

16811F-1



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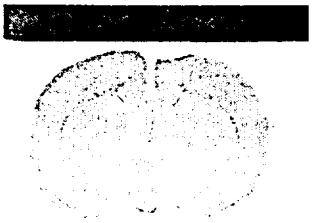

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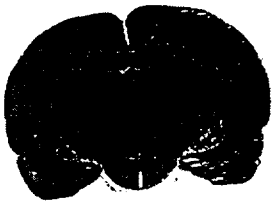

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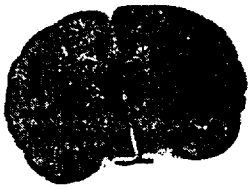

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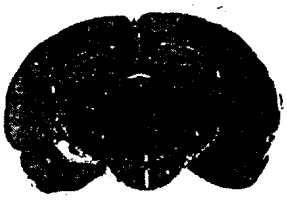

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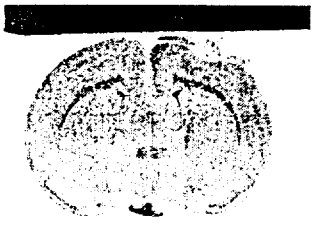

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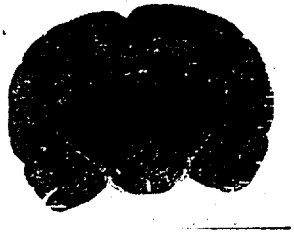

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16817F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats

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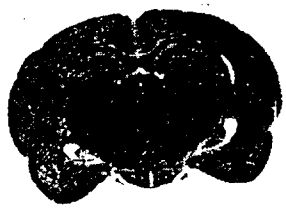

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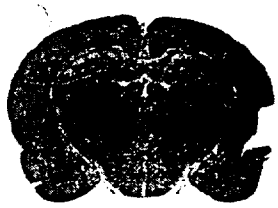
16818F-4

16820F-1



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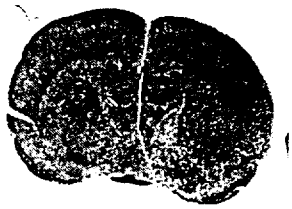

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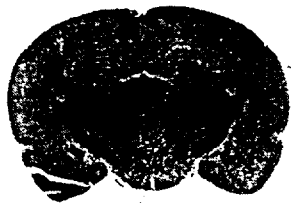

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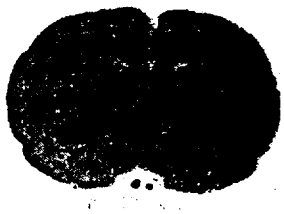

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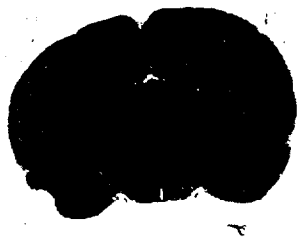

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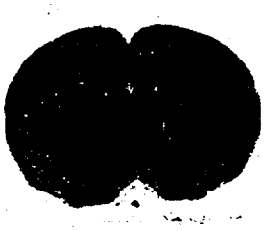
16823F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats



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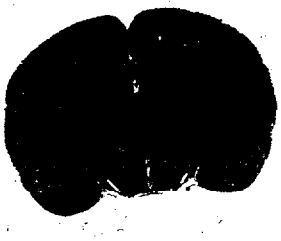

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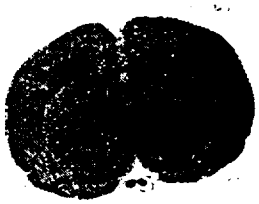

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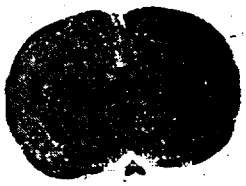
16826F-4



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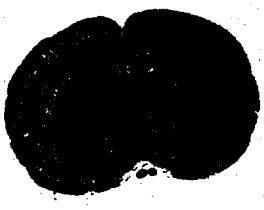

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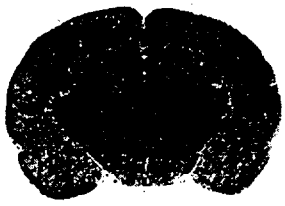

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16828F-4

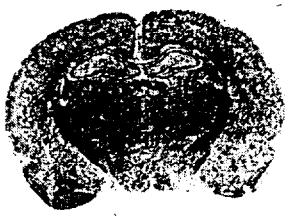
Study 1416-003 – Contact Sheets for Day 22 Female Rats

16830F-1

16830F-2

16830F-4

16831F-1



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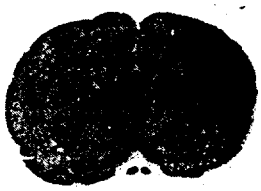
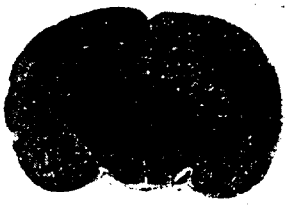


16833F-1

16833F-2

16833F-4

16834F-1



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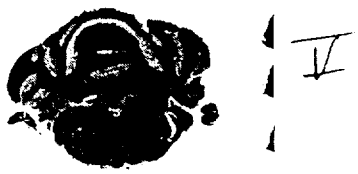
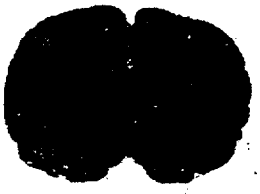


16834F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats

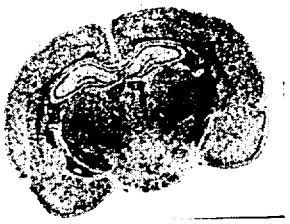


16836F-1

16836F-2

16836F-4

16837F-1



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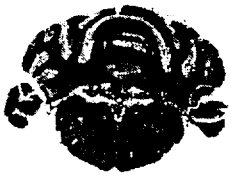

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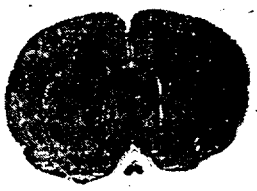
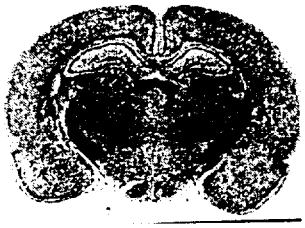


16839F-1



16839F-2

16839F-4

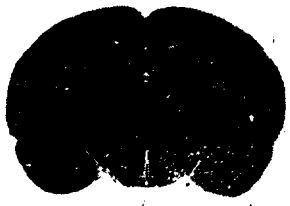
16840F-1

16840F-2

16840F-4

Study 1416-003 – Contact Sheets for Day 22 Female Rats

16842F-1



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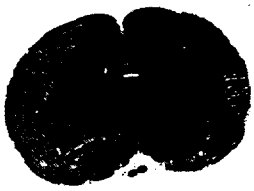

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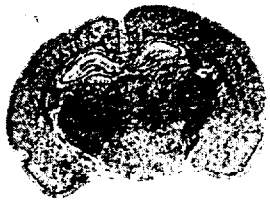

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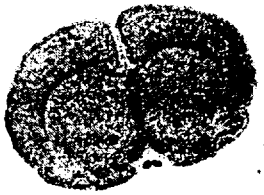

16844F-1

16844F-2

16844F-4